



S2 Fig. Mapping of 2'O-methylguanosines (Gm) and 2'O-methylcytidines (Cm) of 18S rRNA using snoRNA deletion mutants. To corroborate the location of residues mapped by mung bean nuclease assay, snoRNA deletion mutants for the respective ribose methylations were used. A specific loss of peak corresponding to the respective modification in a deletion mutant validated the precise location of the modified residue. Overlaid RP-HPLC chromatograms of the MBN protected fragments containing (A) Gm562 isolated from wild type (WT) and Δ sno40 deletion mutant, (B) Gm1126 isolated from WT and Δ sno41 deletion mutant, (C) Gm1271 isolated from WT and Δ sno40 deletion mutant, (D) Gm1428 isolated from WT and Δ sno56 deletion mutant, (E) Gm1572 isolated from WT and Δ sno57 deletion mutant. To map and calculate the extent of each Cm modification in 18S rRNA, we deleted corresponding snoRNAs and calculated the contribution of individual peak to the total Cm peak area. F) Overlaid chromatograms of 18S rRNA derived nucleosides, isolated from isogenic WT, Δ sno70, and double mutant Δ sno70 Δ sno79. To validate the precise location of ac⁴C 1773, a rDNA point mutant was used where C1773 was exchanged with G in a plasmid-borne copy of 35S rDNA transcribed under the native promoter in a strain where the genomic rDNA was deleted. Exchange of C1773 to G led to 50% reduction in the amount of ac⁴C derived from 18S rRNA. (G) Overlaid chromatograms of isogenic WT and C1773G rDNA point mutant.